

IN THE CLAIMS

Please replace the pending claims with the following amended claims. In accordance with the newly instituted revised amendment format, new material to be added to the claims is shown as underlined, while material to be deleted is shown as ~~struck through~~.

1. (Twice amended) A method of detecting the presence of an intracellular analyte in one or more cells by flow cytometry, the method comprising:
- a) ~~fixing and permeabilizing said cells;~~
 - b) ~~catalyzing the deposition of tyramide in said cells comprising said intracellular analyte by contacting the fixed and permeabilized cells with an antibody or fragment thereof that specifically binds said intracellular analyte, wherein said antibody is directly or indirectly bound to an enzyme that, in the presence of substrate for said enzyme and tyramide, catalyzes the deposition of tyramide in said cells comprising said intracellular analyte, and contacting the fixed and permeabilized cells with tyramide and a substrate for said enzyme;~~
 - c) ~~contacting said cells with a detectable label that directly or indirectly binds to tyramide, whereby said cells comprising said intracellular analyte are specifically labeled; and~~
 - d) ~~detecting a signal from said cells comprising said detectable label using a flow cytometric device, wherein said signal indicates the presence of said intracellular analyte, and wherein said signal is at least 10-fold greater than a signal obtainable by standard flow cytometry methods using in which an immunoglobulin that does not specifically bind said intracellular analyte, and that is isotype/subtype matched to the antibody or fragment thereof of step (b) is used nonspecific immunoglobulin as a negative control.~~
2. (Twice amended) A method of detecting the presence of an intracellular analyte in one or more cells by flow cytometry, the method comprising:

a) fixing and permeabilizing said cells;

b) catalyzing the deposition of tyramide conjugated to a detectable label in said cells comprising said intracellular analyte by contacting the fixed and permeabilized cells with an antibody or fragment thereof that specifically binds said intracellular analyte, wherein said antibody is directly or indirectly bound to an enzyme that, in the presence of substrate for said enzyme and tyramide, catalyzes the deposition of tyramide in said cells comprising said intracellular analyte, and contacting the fixed and permeabilized cells with tyramide conjugated to said detectable label and a substrate for said enzyme, whereby said cells comprising said intracellular analyte are specifically labeled; and

c) detecting a signal from said cells comprising said detectable label using a flow cytometric device, wherein said signal indicates the presence of said intracellular analyte, and wherein said signal is at least 10-fold greater than a signal obtainable by standard flow cytometry methods using in which an immunoglobulin that does not specifically bind said intracellular analyte, and that is isotype/subtype matched to the antibody or fragment thereof of step (b) is used nonspecific immunoglobulin as a negative control.

3. (Amended) A method according to claim 1 or 2, wherein said signal is at least 20-fold greater than a signal obtainable by said standard flow cytometry methods.

4. (Amended) A method according to claim 1 or 2, wherein said signal is at least 50-fold greater than a signal obtainable by said standard flow cytometry methods.

5. (Amended) A method according to claim 1 or 2, wherein said catalyzing step comprises:

81 (i) incubating the fixed and permeabilized cells with said antibody or fragment thereof ~~a binding partner that specifically binds to said analyte~~, wherein said antibody or fragment thereof ~~binding partner~~ is conjugated to said an enzyme that, in the presence of

substrate for said enzyme and tyramide, catalyzes the deposition of tyramide in said cells comprising said intracellular analyte;

(ii) removing unbound antibody or fragment thereof ~~binding partner~~ from said cells;
and

(iii) contacting bound antibody or fragment thereof ~~binding partner~~ with tyramide and said enzyme substrate, whereby said enzyme catalyzes the deposition of tyramide in said cells comprising said intracellular analyte.

6. (Amended) A method according to claim 5, wherein said antibody or fragment thereof ~~binding partner~~ is incubated with said fixed and permeabilized cells in a medium comprising at least about 50% serum.

7. (Reiterated) A method according to claim 6, wherein said serum is fetal bovine serum.

8. (Reiterated) A method according to claim 7, wherein said medium comprises at least about 95% fetal bovine serum.

9. (Reiterated) A method according to claim 8, wherein said medium further comprises about 0.2% saponin.

10. (Reiterated) A method according to claim 1 or 2, wherein said cells are permeabilized in a medium comprising saponin.

11. (Reiterated) A method according to claim 1 or 2, wherein said cells are permeabilized in a medium comprising methanol.

12. (Amended) A method according to claim 5, wherein said bound antibody or fragment thereof ~~binding partner~~ is contacted with tyramide in a medium comprising an aprotic solvent.

13. (Reiterated) The medium of claim 12, wherein said medium comprises at least about 5% of an aprotic solvent selected from the group consisting of acetone, dimethyl sulfoxide, acetonitrile, and dimethyl formamide.

14. (Reiterated) A method according to claim 1 or 2, wherein said detectable label is a fluorochrome.

15. (Reiterated) A method according to claim 14, wherein said fluorochrome comprises a fluorescent molecule selected from the group consisting of fluorescein, phycoerythrin, CY5, allophycocyanine, Texas Red, Peridenin chlorophyll, and cyanine.

16. (Reiterated) A method according to claim 5, wherein said enzyme is selected from the group consisting of hydrolases, peroxidase, oxidase, esterases, glycosidases and phosphatases.

17. (Reiterated) A method according to claim 5, wherein said enzyme is horseradish peroxidase.

18. (Twice amended) A method according to claim 1 or 2, wherein said catalyzing step comprises:

(i) incubating the fixed and permeabilized cells with a second binding partner that specifically binds said antibody or fragment thereof ~~a first binding partner that specifically binds to said first binding partner~~, wherein said second binding partner ~~comprises an enzyme, wherein said second binding partner is conjugated to said~~ an enzyme that, in the presence of substrate for said enzyme and tyramide, catalyzes the deposition of tyramide in said cells comprising said intracellular analyte;

(ii) removing unbound second binding partner from said cells; and

(iii) contacting bound second binding partner with tyramide and said enzyme substrate, whereby said enzyme catalyzes the deposition of tyramide in said cells comprising said intracellular analyte.

19. (Reiterated) A method according to claim 18, wherein said second binding partner is an immunoglobulin-enzyme conjugate.

20. (Reiterated) A method according to claim 19, wherein said second binding partner is incubated with said fixed and permeabilized cells in a medium comprising at least about 50% serum.

21. (Reiterated) A method according to claim 20, wherein said serum is fetal bovine serum.

22. (Reiterated) A method according to claim 12, wherein said medium comprises at least about 95% fetal bovine serum.

23. (Amended) A method according to claim 19, wherein said immunoglobulin-enzyme conjugate is immunoglobulin-peroxidase, immunoglobulin-hydrolase, immunoglobulin-oxidase, immunoglobulin-glycosidase and immunoglobulin-phosphatase.

24. (Reiterated) A method according to claim 23, wherein said immunoglobulin-enzyme conjugate is immunoglobulin-horseradish peroxidase.

25. (Reiterated) A method according to claim 1 or 2, wherein said one or more cells are one or more mammalian cells.

26. (Reiterated) A method according to claim 25, wherein said one or more mammalian cells are selected from the group consisting of basal cells, epithelial cells, erythrocytes, platelets, lymphocytes, T-cells, B-cells, natural killer cells, granulocytes, monocytes, mast cells, Jurkat cells, neurocytes, neuroblasts, cytomegalic cells, dendritic cells, macrophages, blastomeres,

endothelial cells, HeLa cells, tumor cells, interstitial cells, Kupffer cells, Langerhans' cells, Langhans cells, littoral cells, tissue cells, adipose cells, CHO cells, KFL9, and K562 cells.

27. (Reiterated) A method according to claim 1 or 2, wherein said one or more cells are cultured cells.

28. (Reiterated) A method according to claim 1 or 2, wherein said intracellular analyte is selected from the group consisting of intracellular cytokines, antigens, viral antigens, nuclear antigens, cytoplasmic antigens, organellar antigens, enzymes, cytoskeletal molecules, glycolipids, lipids, glycans, chaperones, RNA, DNA, messenger RNA, ribosomal RNA, signal transduction proteins, and structural proteins.

29. (Reiterated) A method according to claim 1 or 2, wherein said intracellular analyte is not a natural component of said one or more cells.

30. (Amended) A method according to claim 1 or 2, wherein said intracellular analyte cannot be detected by said standard flow cytometry methods.

31. (Reiterated) A method according to claim 1 or 2, wherein said one or more cells are obtained from a patient.

32. (Amended) A method according to claim 31, wherein the presence of said intracellular analyte is correlated to a diagnosis of a disease in said patient.

33. (Reiterated) A kit for performing a method according to claims 1 or 2.

34-61 (Previously cancelled)